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Cell Surface: Transfer of Cellular Adhesive Properties from Cell to Cell by Induced Membrane Alterations (38496)

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(Introduced by A. L. Jones)

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The efforts of several laboratories have recently been directed toward the establishment and measurement of biological membrane fluidity. Experiments have been performed to measure lateral diffusion (1) and rotational motion (2, 3) within biological membranes and in lipid bilayers (4, 5). The topography of biological membranes has also been investigated by several laboratories using a variety of markers. The results of these studies for the most part agree with the cell membrane model proposed by Singer and Nicholson (6). The agglutination of cells induced by plant lectins and the distribution of lectin-binding sites on cell surfaces in normal and transformed cells, have suggested models by which lectin-induced cell aggregation may occur (7, 8).

During some recent studies on the surface properties of bone marrow-derived lymphocytes and thymus-derived lymphocytes using covalently-coupled Sepharose–Concanavalin A, we noticed that many cells which had aggregated around the Sepharose–ConA beads were present in multilayers of cells rather than in monolayers. The results reported herein offer evidence for the fluidity of the lymphocyte surface membrane and suggest that induced lateral movement of components on the surface membrane of cells may occur during agglutination reactions, during cell aggregation to form tissues, and perhaps may be associated with other cell surface phenomena.

Methods and Materials. Concanavalin A covalently coupled to Sepharose 4B was prepared according to the procedure of Lloyd (9) using ConA purified by the method of Agrawal and Goldstein (10). Bone marrow lymphocytes and thymic lymphocytes were prepared from the respective tissues of 4- to 8-week old male BDF_1 mice. Cells were washed thoroughly with Eagles MEM, passed through nylon mesh to remove any cell aggregates and finally suspended in Eagles MEM at 1×10^7 cells ml. Aggregation of the cells to Sepharose–ConA was induced in the following way. An aliquot of a cell suspension (0.5–1 ml) was placed in a small glass tube, centrifuged at 1000 *g* for 10 min and the supernatant solution decanted. To the cells was added 0.5 ml of a suspension of Sepharose–ConA (0.75 g of moist gel in 6 ml of Eagles MEM) and the cells resuspended gently. The suspended cells and beads were either allowed to settle for 1 hr at 25° or were centrifuged for 5 min at 1000 *g*. The pellet was gently resuspended by holding the tube at a 45° angle and rotating it slowly. Experiments with an inhibitor of ConA binding consisted of performing the same procedure except that the Sepharose–ConA suspension was made 33 mM with α-methyl-D-mannoside.

Results and Discussion. Using either of the above procedures, cell-aggregated Sepharose–ConA beads typically appeared as shown in Fig. 1a. Over 95% of the cells added were attached to the beads. This was determined by counting the free cells in suspension with a hemocytometer after allowing the beads to settle for 3 min. The number of free cells was then compared to the number of cells in suspension of control tubes with unmodified Sepharose. Cells were found to be aggregated in multilayers rather than in a single layer of cells surrounding the beads. These results were unexpected since the cells occupying the layers not in direct contact with the beads presumably were not adhering to other cells via ConA. It is unlikely that there was any appreciable ConA which was unattached to the Sepha-

1. Matsumoto, H., and Strong, F. M., Arch. Biochem. Biophys. **101,** 299 (1963).
2. Grant-Whiting, M., Econ. Bot. **17,** 4, 271 (1963).
3. Hall, W. T., and McGavin, M. D., Pathol. Vet. **5,** 26 (1968).
4. Laqueur, G. L., Mickelsen, O., Whiting, M. G., and Kurland, L. T., J. Nat. Cancer Inst. **31,** 919 (1963).
5. Hirono, I., and Shibuya, C., Nature (London) **216,** 1311 (1967).
6. Hirono, I., and Shibuya, C., Proc. Soc. Exp. Bio. Med. **131,** 593 (1969).
7. Shimada, M., and Langman, J., Teratology **3,** 119 (1969).
8. Spatz, M., and Laqueur, G. L., Proc. Soc. Exp. Bio. Med. **129,** 705 (1968).
9. Spatz, M., Ann. N.Y. Acad. Sci. **163,** 848 (1969).
10. Sanger, V. L., Yang, M., and Mickelsen, O., Fed. Proc. **31,** 1524 (1972).
11. Jones, M., Amer. J. Pathol. **61,** 6a (1971).
12. Hirano, A., and Jones, M., Fed. Proc. **31,** 1517 (1972).
13. Hirano, A., Dembitzer, H. M., and Jones, M., J. Neuropathol. Exp. Neurol. **31,** 113 (1972).
14. Jones, M., Gardner, E., and Yang, M., Amer. J. Pathol. **66,** 3a (1972).
15. Jones, M., Sweeley, C., and Yang, M., Fed. Proc. **31,** 1512 (1972).
16. Jones, M., Sweeley, C., and Yang, M., J. Neuropathol. Exp. Neurol. **31,** 191 (1972).
17. Jones, M., Yang, M., and Mickelsen, O., Fed. Proc. **31,** 1508 (1972).
18. Jones, M., Mickelsen, O., and Yang, M., Progr. Neuropathol. **2,** 91 (1973).

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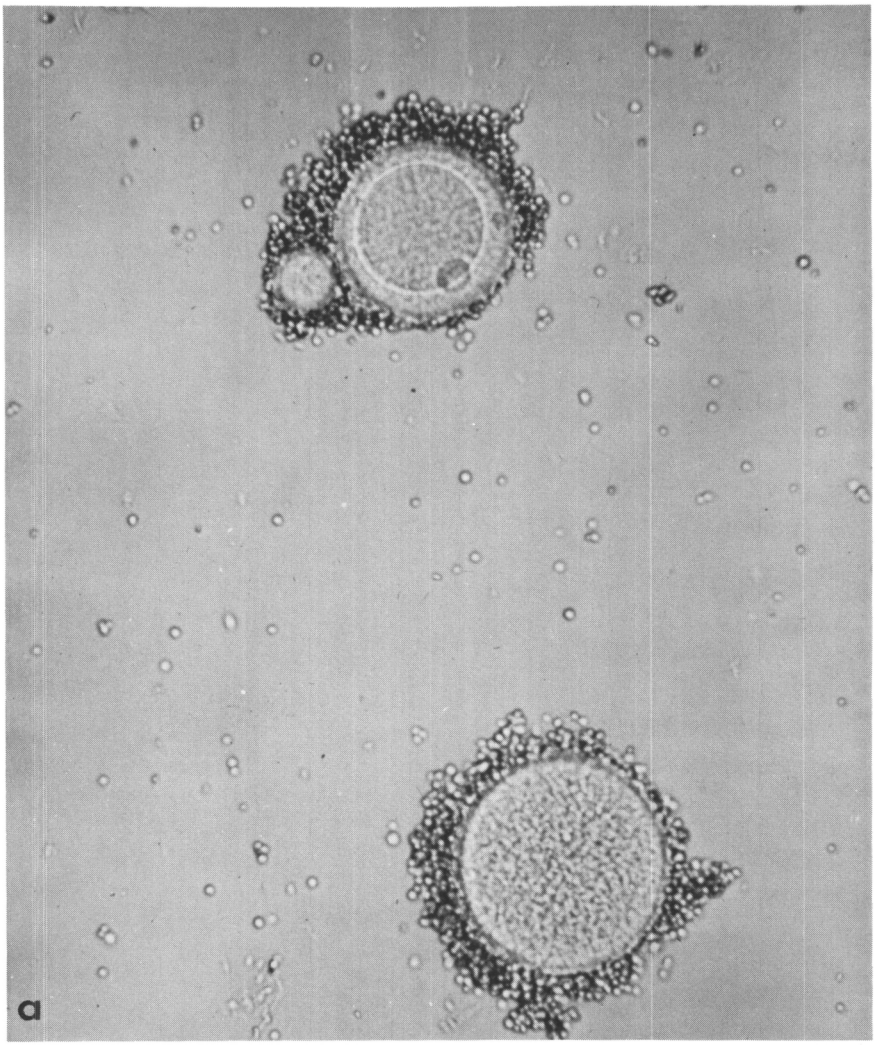

FIG. 1a. Thymic cells aggregated on Sepharose–ConA beads. Aggregation was produced as described in the text. The Sepharose beads are the larger spheres. (Magnification ×400). b. Thymic cells and Sepharose–ConA beads in the presence of 33 m*M* α-methyl-D-mannoside. (Magnification ×400).

rose causing externally located cells to aggregate. This conclusion was reached for the following two reasons: (a) The Sepharose–ConA was washed thoroughly after preparation and just prior to the aggregation experiments; and (b), when the Sepharose–ConA cell aggregates were resuspended carefully, the only clumped cells seen were those associated with the beads. When α-methyl-D-mannoside was present during the aggregation step, none of the thymic cells were associated with the beads (Fig. 1b). When bone marrow cells were used, however, even with α-methyl-D-mannoside present, many of the cells formed aggregates with the beads.

It seems from these observations that not all of the cells which bound to Sepharose–ConA were linked to the beads directly by ConA. It was concluded from recent studies on the agglutination of mouse embryo fibroblasts (7) that ConA-mediated cell agglutination requires lateral movement of ConA receptor sites within the surface membrane. Other recent studies have shown that there is lateral movement of surface antigens and receptors in lymphocytes (8, 11, 12). The present studies suggest that in

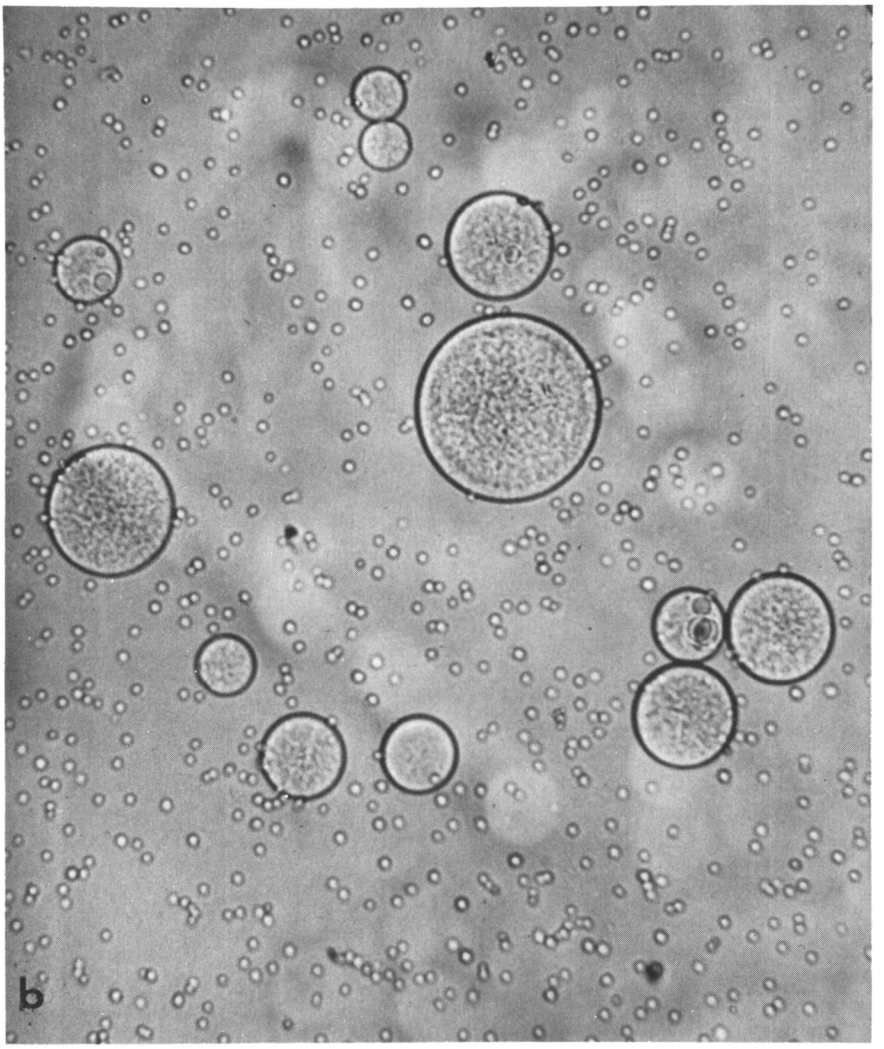

FIGURE 1b.

addition to a lateral movement of ConA-binding sites and perhaps a concentration of these sites on the cell surface adjacent to Sepharose-bound ConA, concomitant alterations in cell surface properties occur in lymphocytes. These alterations, perhaps lateral charge polarization or the aggregation of other groups on the cell surface, may elicit complimentary changes in an adjacent cell, allowing the two cells to aggregate. The induced lateral movement of surface sites may be transferred from cell to cell over several cell layers. The aggregation observed in the present study with lymphocytes appears to be the same phenomenon as that observed by Chipowsky *et al* (13) with fibroblasts but probably occurs by a different mechanism since ConA contains no galactose (14). We suggest that this polarization transference phenomenon may account for lectin and antibody-induced cell agglutination as well as for tissue formation. The ability of cells to transfer surface properties to adjacent cells may allow cells to be agglutinated without cell-agglutinin-cell complexes to be formed throughout the aggregate. In addition the transference phenomenon may account for the arrangement of cells in tissue resulting in specific membrane surfaces with defined enzymatic and structural properties suitably oriented.

Summary. Lymphocytes formed aggre-

gates around Sepharose beads to which Concanavalin A had been coupled. Many of these aggregates consisted of multilayers of cells. The cell–cell interactions distal to the beads appeared to be a result of an induced membrane change at the cell-Sepharose bead interface.

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1. Edidin, M., and Fambrough, O., J. Cell Biol. **57,** 27 (1973).
2. Cone, R. A., Nature New Biol. **236,** 39 (1972).
3. Brown, P. N., Nature New Biol. **236,** 35 (1972).
4. Devaux, P., and McConnell, H. M., J. Amer. Chem. Soc. **94,** 4475 (1972).
5. Hubbell, W. L., and McConnell, H. M., J. Amer. Chem. Soc. **93,** 314 (1971).
6. Singer, S. J., and Nicolson, G. L., Science **175,** 720 (1972).
7. Noonan, K. O., and Burger, M. M., J. Cell Biol. **59,** 134 (1973).
8. Loor, F., Exp. Cell Res. **82,** 415 (1973).
9. Lloyd, K. O., Arch. Biochem. Biophys. **137,** 460 (1970).
10. Agrawal, B. B. L., and Goldstein, I. S., Biochim. Biophys. Acta **133,** 376 (1967).
11. Raff, M. C., and DePetris, S., Fed. Proc. **32,** 48 (1973).
12. Karnovsky, H. J., and Unanue, E. R., Fed. Proc. **32,** 48 (1973).
13. Chipowsky, S., Lee, Y. C., and Roseman, S., Proc. Nat. Acad. Sci. U.S.A. **70,** 2309 (1973).
14. Edelman, G. M., Cunningham, B. A., Reeke, G. N., Jr. Becker, J. W., Waxdal, M. J., and Wang, J. L., Proc. Nat. Acad. Sci. U.S.A. **69,** 2580 (1972).

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